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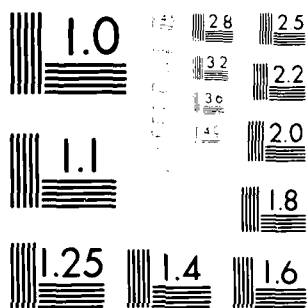
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significantly reduced run times (108, 67 and 54 min), more rapid accumulations of metabolic heat (.04, .057 and .062°C/min) and much higher mortalities (50, 69, and 50%) despite smaller fluid losses (5.2, 3.7, or 4.2%). Although the mechanisms responsible for these varying thermoregulatory responses to exercise-induced exhaustion are not fully understood, the present data indicate that the rate of fluid loss, body composition, surface area to mass ratio and age are important variables.

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EFFECT OF AGE, WEIGHT AND METABOLIC
RATE ON ENDURANCE, HYPERTHERMIA
AND HEATSTROKE MORTALITY

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Effect of Age and Weight on Heatstroke Mortality

ABSTRACT

Since exercise performance is related to age, body weight, and metabolic efficiency, this investigation was designed to determine in rats the effects of these variables on endurance, thermoregulation and heatstroke mortality at a relatively mild environmental temperature, (26°C). The results indicate that light rats (250g) are able to run (11m/min, 6° incline) longer (221min), accumulate less metabolic heat (.019 C°/min) and thereby experience low heatstroke mortality (20%) despite a large amount of fluid loss (6.1%). This is in contrast to heavier and older rats (350g-500g) which demonstrated significantly reduced run times (108, 67 and 54 min), more rapid accumulations of metabolic heat (.04, .057 and .062°C/min) and much higher mortalities (50, 69 and 50%) despite smaller fluid losses (5.2, 3.7, or 4.2%). Although the mechanisms responsible for these varying thermoregulatory responses to exercise-induced exhaustion are not fully understood, the present data indicate that the rate of fluid loss, body composition, surface area to mass ratio and age are important variables.

Key Words: metabolic rate, skin temperature, rectal temperature, age, weight, endurance capacity, rat

Introduction

Moderate exercise or exhaustive work may lead to progressive dehydration as well as energy depletion (9). The dehydration and energy deficits contribute to the deterioration of physiological functions such as temperature regulation and cardiovascular compensation (2,12,14) which could not only impair performance but ultimately culminate in heatstroke and death. Like other disorders which cannot be linked to a primary causative factor, heatstroke is multifaceted and is usually, but not always, associated with several variables such as age (20), body weight (21), maximal oxygen consumption (23,24), ambient temperature (17,21) or level of hydration (19).

Since heatstroke frequently occurs during athletic events and rigorous military training (7), our attention has been drawn to exercise per se as a contributing factor in its etiology. In fact, heatstroke has often been reported in highly motivated individuals including athletes or soldiers engaged in heavy exertion even on relatively cool (26°C) days (18). Since exercise performance is related to age, body weight, ambient temperature, and metabolic efficiency, this investigation was designed to determine the effects of these variables on endurance, hyperthermia and heatstroke mortality in an exercising rat model (8) for human hyperthermic injury.

Materials and Methods

Experimental animals. Male Sprague-Dawley rats (CD-1, Charles River Breeding Laboratories, Wilmington, MA) were used in all experiments. Animals were purchased at 150 ± 20 g ($\bar{X} \pm$ SEM), so that adequate time was available for the animals to acclimate to laboratory conditions before their targeted weights were achieved. All animals were caged individually in an environmental chamber (3x3x2m) maintained at 26°C and 40% relative humidity; makeup air was replaced at a rate equivalent to 1.4 room volumes per hour. Rats were fed a standard diet (Ralston Purina Rodent Chow #5001) and water ad libitum.

Animals were divided into four groups based on approximate weight and age. Since growth rate is not linear after 500 g, this was selected as the upper weight limit. In order to maintain the old animals at 500 g, we matched their caloric intake to their calculated daily energy expenditure (approx 65Kcal/Kg).

Experimental Design. When the animals had achieved the appropriate experimental weight and/or age, food, but not water, was removed at approximately 1700h on the day prior to the exercise. This regimen assured proper hydration and reduced the variability associated with feeding and excretion. In addition, urine loss during exercise was minimized by temporarily restraining the animals prior to exercise, a procedure which generally elicited micturition.

On the morning of a treadmill-run animals were randomly selected from the four groups, weighed, and placed in a metabolic chamber. After an appropriate acclimation period oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) and respiratory quotient (RQ) were measured. The airtight plexiglass chamber contained inflow and outflow openings with Luer-lock connections that permitted sampling by an online system directly connected to O_2 (Applied Electrochemistry S-3A) and CO_2 (Beckman LB-2) analyzers. Calibration gases for these instruments were analyzed at monthly intervals by mass spectrometry. Room air was drawn through the chamber by a variable Roller Flex pump; humidity was removed before entering and leaving the chamber by means of Drierite traps. Airflow was adjusted by means of a flow meter. Following measurement of metabolic rate, animals were removed from the chamber, restrained and were instrumented for measuring core (T_{co}) and tail-skin (T_{sk}) temperatures. T_{co} (6.5 cm) and T_{sk} (midlength) were measured during the exercise using platinum resistance thermometers (13). The temperature acquisition system consisted of a Hewlett-Packard (HP) 9825 computer, IEEE-488 interface bus cables, an HP 34555A Digital Volt/Ohm Meter, and an HP-3495A Scanner/ Multiplexer.

After instrumentation animals were exercised to exhaustion at an environmental temperature of $26^{\circ}C$. The motor-driven treadmill was similar to the one described by Pattengale and Holloszy (15). Rats exercised on a 6° incline at 11m/min with a 2 min rest

period after 20 and 40 min of work. Exhaustion was achieved under a shock-avoidance contingency, and was defined as that point at which rats could not keep pace, and when placed on their backs, were unable to right themselves. After exhaustion, temperature monitoring was continued at 26°C for 20min. The animals were then returned to their cages (26°C) for 24h with water but no food.

Statistical analyses were performed with BMDP statistical software on a VAX computer system. The data were examined for skewness and kurtosis, and for verification of equal variances within groups. One way analysis of variance was performed, and significance ($P < .05$) was determined by Tukey's post hoc test. In addition linear regression analyses was performed to determine significance of correlation coefficients.

Results

The basal metabolic rate did not change significantly with age or body weight when expressed as the $2/3$ power of body weight (Table 1). RQ was similar (.81) in all groups (data not shown).

Exercise-induced dehydration, expressed as the total weight loss, appeared to increase with age ($P < .05$) (Table 2). However, when expressed as a percentage of initial body weight, the lighter, younger rats (Group 1) lost a greater proportion of their body water despite a lower rate of loss (G/min) ($P < .05$, Table 2). This was due to their significantly longer run time. In fact, it is interesting to note a significant inverse correlation between weight loss/min and endurance ($P < .001$). This relationship may be age and not weight related since Group 4 (old and heavy) manifested a significantly ($P < .05$) increased rate of water loss when compared to Group 3 (Table 2).

The data in Table 3 demonstrate the thermoregulatory responses to exhaustive exercise and suggests that exhaustion occurs at a T_{re} of approximate 42°C . Heat dissipation and thermoregulation appear more efficient in the light animals as manifested in greatly reduced heating rates ($P < .05$) prior to exhaustion and also in more rapid post-run cooling rates. The ability to thermoregulate is decreased as the weight and/or age increased as reflected in elevated heating rates and lower cooling rates.

Representative heating curves of T_{co} (top line) and T_{sk}

(bottom line) for survivors and fatalities during exercise are illustrated in Figure 1 and 2. Several observations should be noted: 1) younger and lighter rats require more time to achieve threshold-heatstroke temperature of 40.4°C , 2) whether the rat survives or dies, exhaustion is associated with a divergence of T_{co} and T_{sk} , and 3) during the post-run cooling period, eventual fatalities continued to exhibit divergence of T_{re} and T_{sk} while the survivors displayed a converging pattern.

The severity of exercise-induced hyperthermia was calculated as an area in degree-minutes above a baseline T_{co} of 40.4°C . In the exhausted rat a T_{co} of 40.4°C is the minimum T_{co} which resulted in death within 24h(8). The relationship between this heat storage and mortality is presented in Table 4. The data indicate that the manner in which thermal area is accumulated as well as the total heat storage differs in the four groups. For example, Groups 1 & 2 accumulated more thermal area during exercise while Groups 3 and 4 accumulated more post-exercise. Secondly, the data confirm the direct correlation ($r=.85$) between the accumulation of total thermal area and the % heatstroke mortality.

Discussion

This investigation was designed to evaluate the effects of metabolic rate, age and weight on exercise duration, hyperthermia and heatstroke mortality; a secondary purpose was to investigate the hypothesis that heatstroke levels of hyperthermia may occur

during exhaustive exercise even under relatively mild conditions. The results suggest that hyperthermic exhaustion may seriously affect endurance studies conducted at or near room temperature. Previous studies using the rat heatstroke model have demonstrated that exercise-induced hyperthermia even at moderate temperatures is more injurious than passive hyperthermia (14). However, although the rat has been used extensively as a model to elucidate physiological and thermoregulatory mechanisms, there are limited data available on the effects of metabolic rate, weight and age on performance, thermoregulatory efficiency and heatstroke.

Historically, metabolic rate may be expressed in a variety of units which are ordinarily selected for a particular experimental situation. We have expressed metabolic rate as the $2/3$ power of body weight thus minimizing the effects of body mass on this variable. This expression is recommended for use in investigations when considerable differences exist in body mass and age (4). Additionally, the use of this unit to express metabolic rate is appropriate for information concerning heat exchange or cooling. Although there is no relationship between the resting metabolic rate (VO_2) and mortality or heating rate, a correlation may exist between the resting metabolic rate and the cooling rate. The lower metabolic rate of the heavy and heavy older rats may be secondary to or result from a decreased ability to dissipate metabolic heat as reflected in a slower cooling rate post-exercise (Table 1 and 3).

Treadmill exercise had markedly different effects on physiological responses and thermoregulation in the four groups. Performance and thermoregulation was far superior in Group 1, and this is consistent in this group with the low rate of weight (water) loss. Cardiovascular integrity persists in this group and sustains tail blood flow resulting in a significant increase in total heat loss. Rand et al (16) have shown that a marked increase in heat loss occurs at an environmental temperature of about 25°C and this increase is correlated with increased tail blood flow. Further, they have estimated that at 30°C, blood flow to the tail increases 15 to 20 fold. The heat loss after vasodilation measured calorimetrically was about 60 cal/min/100 ml of tail. Thus, during heat stress the tail is able to dissipate about 17% of the total heat production of the rat. Therefore, the maintenance of cardiac output by fluid retention and constriction of other vascular beds (10) maintains tail blood flow for heat dissipation, reduced T_{co} , and optimal performance. It appears that Groups 2, 3, and 4 are progressively worse in making this homeostatic compensation.

Although we did not measure $\dot{V}O_2$ max, it has been reported to decline with age and weight (4). Thus, performance decrements in the heavy and older heavy groups were anticipated and observed. Since all groups are running at the same treadmill speed, the older and heavier rats are running at a higher percentage of their reduced $\dot{V}O_2$ max. A greater dependency on anaerobic

metabolism could, in part, result in greater heat production, rate of heat gain and storage. Therefore, the decreased aerobic capacity and the high rate of fluid loss may have resulted in reduced cardiac output, a decline in performance, and the high percent heatstroke mortality in the older and heavier groups. The apparent paradox of tail vasodilation for heat dissipation in the face of functional hypovolemia may contribute to exhaustion, hyperthermia, and increased heatstroke mortality.

During exercise T_{co} increases followed by a sudden increase in T_{sk} reflecting the onset of vasodilation (22). Thus, during the initial stages of exercise, cardiovascular responses provide compensation to the moderate reduction in vascular resistance. However, just prior to exhaustion, there occurs a characteristic divergence between core and tail temperatures indicating an impending circulatory crisis. Barger et al (3) have shown that cutaneous blood flow decreases drastically as exhaustion nears during exercise in the heat. Cardiovascular compensation to maintain cardiac output now jeopardizes the ability to dissipate heat from the skin. This compensatory response is seen in all four groups prior to exhaustion whether the animals survived or died (Fig 1 and 2). Although exhaustion occurred in the four groups at approximately 42°C T_{co} , the interval of elevated T_{co} may be the critical factor in determining ultimate mortality. The data in this study support this since thermal load, as indicated by degree-minutes above 40.4°C , correlates well with

percent mortality. In addition, since thermal area is calculated from both the elevation and duration of T_{co} above 40.4°C , which is the threshold level of body temperature for heatstroke as estimated by Leithead and Lind (11), the temperature at which an animal thermoregulates will determine its thermal load. For example, the light group was able to thermoregulate at a temperature below 40.4°C , enabling longer run times with little accumulated thermal area. When fluid loss became critical, thermoregulation deteriorated at a moderate pace resulting in the accumulation of thermal area until exhaustion. Since the light group retained cooling capacity, thermal area was minimal during this cooling period. In comparison, Group 2 (heavier and older) thermoregulated at or above 40.4°C and thus accumulated greater thermal area. Since this group had a significantly higher EOR T_{co} , more thermal area was accumulated despite similar cooling rates. Therefore, it appears that the ability to thermoregulate at a temperature below the threshold for heatstroke is critical in maximizing performance, decreasing thermal load and hence reducing heatstroke mortality.

The thermal area data from Group 3 are in close agreement with the data of Hubbard et al (8). The data in Figure 2 indicate that Group 3 attains the threshold temperature for heatstroke, 40.4°C , very rapidly, and hence accumulated a small amount of thermal area while on the treadmill. However, its inability to thermoregulate following exercise caused it to

retain much more thermal load during the cooling period (Table 3 & 4). Although Group 1 and Group 3 have similar thermal areas to exhaustion, Group 3 continues to accumulate thermal area during the cooling period resulting in a greater total thermal load and greater mortality.

Finally, age is another variable in determining thermoregulatory efficiency during exercise. The data show that Group 4, the oldest group, had the shortest run time, most rapid heating rate, and accumulated the smallest amount of thermal area to EOR. These responses are probably all related to the rapid rate of weight (water) loss during the exercise. These responses are, so dramatic that, coupled with the slow cooling rate, this group continues to accumulate thermal area during the cooling period (Table 4). Although one would expect the highest mortality in this group, this was not observed. One possible explanation could be the fact that the rapid onset of exhaustion resulting in a small accumulation of thermal area to exhaustion assured reduced exercise stress. Although cooling was depressed, exposure was minimized as evidenced by the smaller amount of total accumulated thermal area, and may have allowed a higher survival rate than anticipated.

In summary, these experiments have compared the effects of age, weight and metabolic rate on exercise duration, hyperthermia and heatstroke mortality. The results indicate: 1) in a relatively mild environment exercise induced exhaustion can

result in a high percent mortality, 2) thermoregulatory efficiency is related to the ability to conserve body water and thereby cardiovascular stability; and 3) the percent heatstroke mortality increases as the weight and age of the rats increase, and is correlated with the heating rate and total accumulation of thermal area. Finally, it is recommended that studies designed to elucidate performance, heatstroke susceptibility, and drug intervention consider the age and weight of the experimental animals and the potentially injurious heat loads which will be generated.

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Disclaimer Statement

The views, opinions and findings contained in this report are those of the authors and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other official documentation.

Statement On Animal Use

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

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TABLE 1

AGE, WEIGHT AND BASAL METABOLIC PARAMETERS

	GROUP			
	1	2	3	4
GROUP	LIGHT	MEDIUM	HEAVY	OLD HEAVY
AGE, WEEKS	8.0 \pm 0.5	12.0 \pm 0.5	16.0 \pm 0.5	30.0 \pm 1.0
WEIGHT, G N=16	237 \pm 3.0	356 \pm 3.0	503 \pm 3.0	504 \pm 3.0
$\dot{V}O_2$, $\mu\text{MOL}/\text{KG}^{2/3}\cdot\text{MIN}$	739 \pm 32	737 \pm 25	688 \pm 22	677 \pm 31

TABLE 2
EFFECT OF EXHAUSTIVE EXERCISE ON PERFORMANCE AND WEIGHT LOSSES

RUN TIME, MIN	221 ± 26	108 ± 10*	67 ± 7*	54 ± 4*†
WORK, KG, MIN	61.0 ± 6.9	42.2 ± 3.6*	36.8 ± 3.0*	29.7 ± 2.4*
WT LOSS, G/MIN	.085 ± .01	.189 ± .01*	.289 ± .02*	.418 ± .04*† ^α
WT LOSS, G	14.5 ± 1.0	18.6 ± 0.9	18.3 ± 1.2	21.0 ± 1.5*
% WT LOSS	6.1 ± 0.4	5.2 ± 0.3	3.7 ± 0.2*†	4.2 ± 0.3*

DATA ARE PRESENTED AS MEANS ± SE. *DENOTES A SIGNIFICANT DIFFERENCE FROM GROUP 1. †SIGNIFICANTLY DIFFERENT FROM GROUP 2. ^αSIGNIFICANTLY DIFFERENT FROM GROUP 3. (P .05)

TABLE 3
THERMOREGULATORY RESPONSES TO EXHAUSTIVE EXERCISE

	GROUP			
	1	2	3	4
EOR CORE TEMP, °C	41.7 ± 0.16	42.3 ± 0.05*	42.0 ± 0.12	41.9 ± 0.06
EOC CORE TEMP, °C	40.3 ± 0.18	40.9 ± 0.18	41.1 ± 0.25*	41.1 ± 0.13*
HEATING RATE, °C/MIN	.019 ± .004	.040 ± .005	.057 ± .005*†	.062 ± .006*
COOLING RATE, °C/MIN	.069 ± .004	.069 ± .007	.045 ± .007	.048 ± .006

DATA ARE PRESENTED AS MEANS ± SE. *DENOTES A SIGNIFICANT DIFFERENCE FROM GROUP 1.
† SIGNIFICANTLY DIFFERENT FROM GROUP 2. (P<.05).

TABLE 4
EFFECT OF EXHAUSTIVE EXERCISE ON ACCUMULATION OF THERMAL EXPOSURE IN DEG.MIN

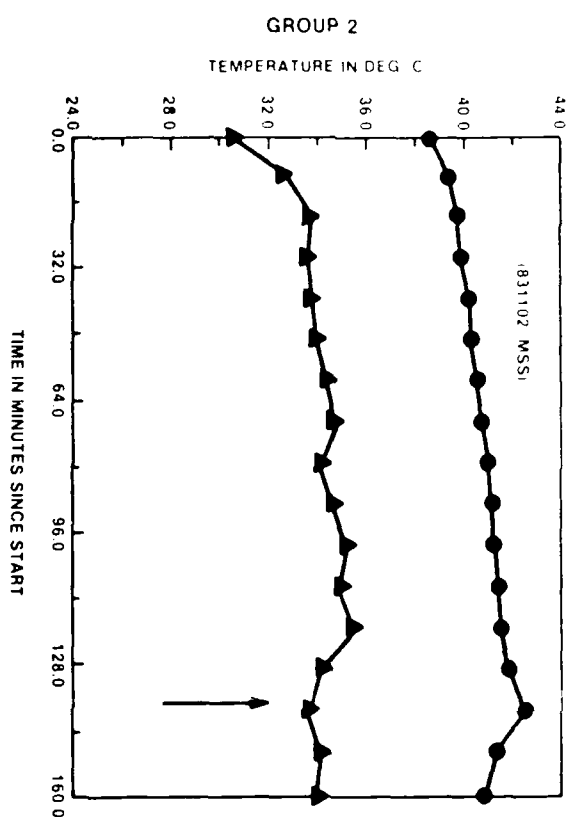
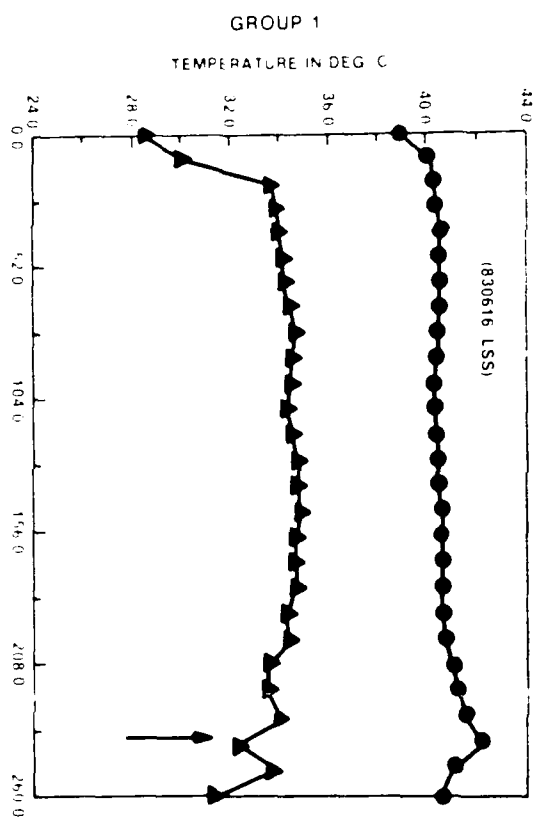
GROUP	(40.4°C TO EOR)	(EOR TO 40.4°C)	TOTAL THERMAL AREA	% MORTALITY
1	33.5 ± 5.8	17.6 ± 6.0	51.0 ± 9.3	20
2	50.4 ± 4.7	24.7 ± 3.0	73.9 ± 6.6	50
3	32.0 ± 4.4	87.4 ± 9.8	120.6 ± 31.2*	69
4	17.6 ± 2.1	41.7 ± 7.0	59.2 ± 12.2	50

DATA ARE PRESENTED AS MEANS ± SE. *DENOTES A SIGNIFICANT DIFFERENCE FROM GROUP 1.
(P < .05) EOR: END OF RUN

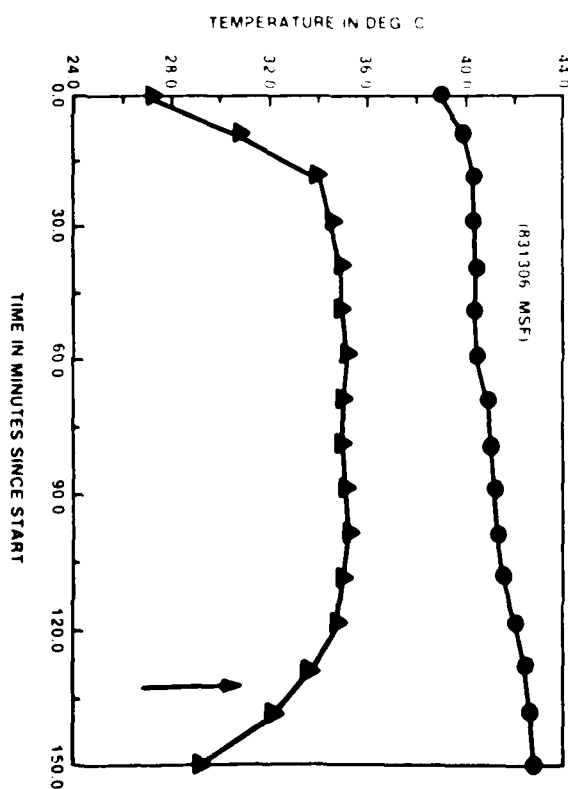
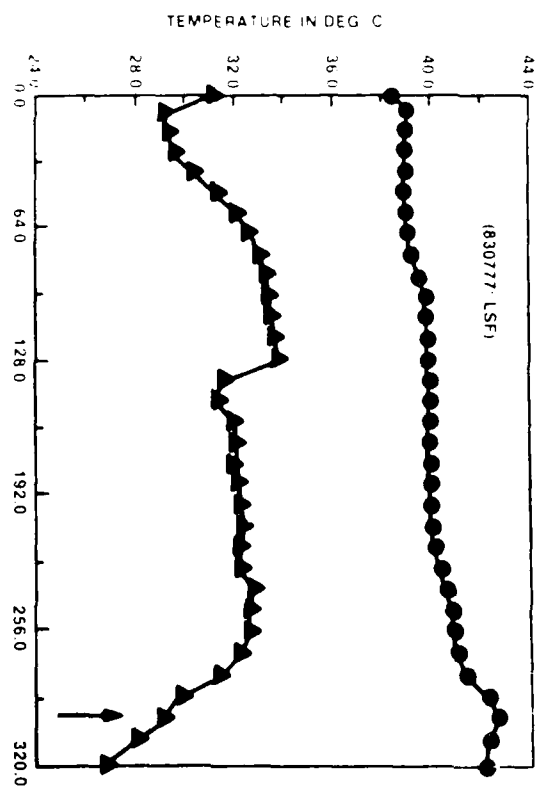
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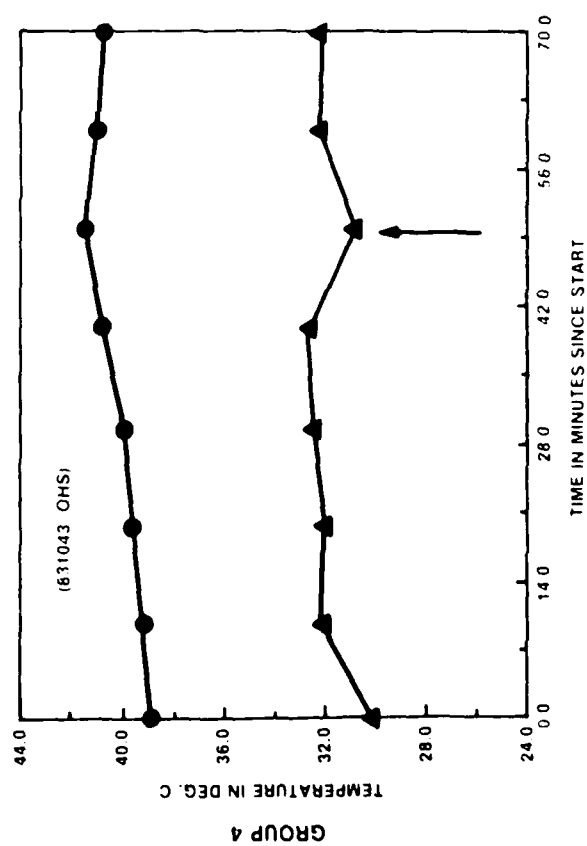
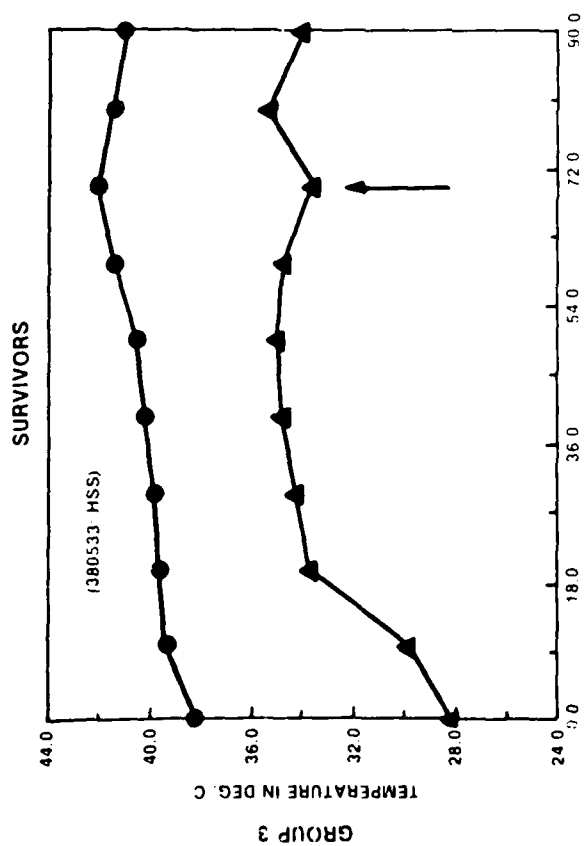
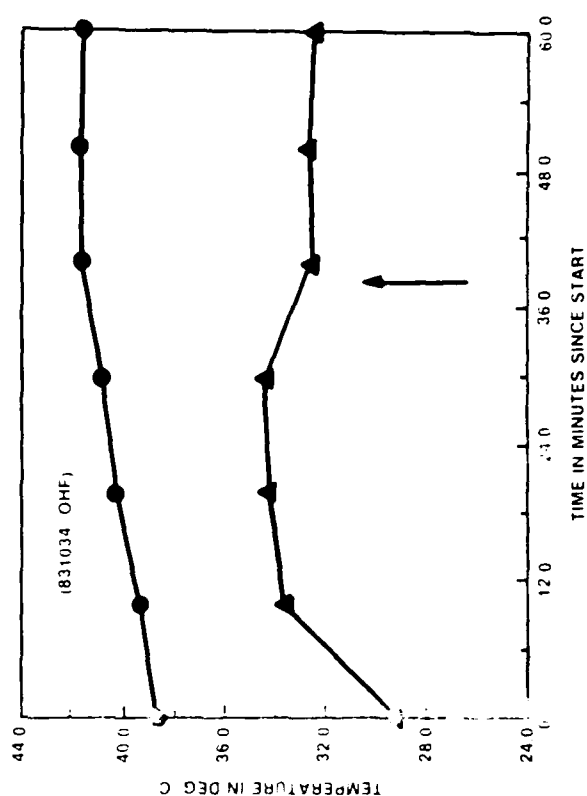
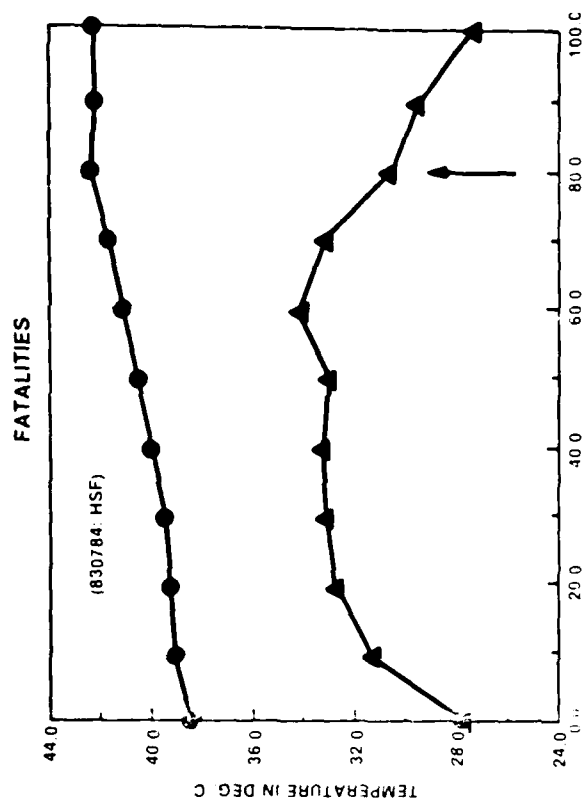
- Fig. 1 Representative heating curves with T_{co} (●) and T_{sk} (▲) plotted against run time for group 1 and 2. Figure is divided into survivors (left panel) and fatalities (right panel). Arrows indicate EOR (end of run) exhaustion.
- Fig. 2 Representative heating curves with T_{co} (●) and T_{sk} (▲) plotted against run time for group 3 and 4. Figure is divided into survivors (left panel) and fatalities (right panel). Arrows indicate EOR (end of run) exhaustion.

SURVIVORS



FATALITIES





GROUP 3

GROUP 4